17 LSD QUANTITATION AND CONFIRMATION BY GCMS

17.1 Summary

17.1.1 LSD and LSD-d_3 (internal standard) are extracted from biological samples using solid phase extraction (SPE) and injected into a GCMS for confirmation and quantitation by selected ion monitoring.

17.2 Specimen Requirements

17.2.1 2 mL of whole blood, biological fluid or tissue homogenate

17.3 Reagents and Standards

17.3.1 LSD, 1 mg/mL

17.3.2 Methadone, 1 mg/mL

17.3.3 Mepivicaine, 1 mg/mL

17.3.4 LSD-d_3 0.1 mg/mL

17.3.5 Methanol

17.3.6 Dichloromethane

17.3.7 Isopropanol

17.3.8 Toluene

17.3.9 Hexane

17.3.10 Isoamyl alcohol

17.3.11 Potassium phosphate buffer solution concentrate (1 M, pH 6.0, e.g. Fisher)

17.3.12 Ammonium hydroxide, concentrated

17.3.13 Acetic Acid

17.4 Solutions, Internal Standard, Calibrators and Controls

17.4.1 Toluene:Hexane:Isoamyl Alcohol (THIA) (78:20:2, v:v:v) Mix 78 mL toluene, 20 mL hexane and 2 mL isoamyl alcohol

17.4.2 Phosphate buffer 0.1 M, pH 6.0: Dilute one volume potassium phosphate buffer solution concentrate with nine volumes of dH_2O.

17.4.3 1 M Acetic acid: Add 100-200 mL dH_2O to a 1 L volumetric flask. Add 57.5mL glacial acetic acid and QS to volume with dH_2O.

17.4.4 Elution Solvent (prepare fresh daily): Dichloromethane/isopropanol/ammonium hydroxide (78:20:2). Mix 78 mL dichloromethane with 20 mL isopropanol. Mix well. In hood, add 2 mL ammonium hydroxide. Mix gently.

17.4.5 LSD calibrator stock solution 2 µg/mL: Pipet 20 µL LSD standard (1 mg/mL) into a 10 mL volumetric flask. QS to volume with methanol.

17.4.6 LSD calibrator working solution 0.2 µg/mL: Pipet 1.0 mL LSD Stock Solution (2 µg/mL) into a 10 mL volumetric flask. QS to volume with methanol.

17.4.7 LSD-d_3 2 µg/mL internal standard solution: Pipet 200 µL LSD-d_3 (1mg/mL) into a 10 mL volumetric flask. QS to volume with methanol.

17.4.8 LSD control solution (0.2 µg/mL):

17.4.8.1 2 µg/mL LSD QC solution: Pipet 20 µL LSD stock (1 mg/mL, manufacturer or lot number different than that used for calibrators) into a 10 mL volumetric flask and QS to volume with methanol.

17.4.8.2 0.2 µg/mL LSD QC solution: Pipet 1.0 mL 2 µg/mL LSD QC solution into 10 mL volumetric flask and QS to volume with methanol.

17.4.9 Mepivicaine solution 25 µg/mL: Pipet 50 µL mepivicaine standard (1 mg/mL) into a 2 mL volumetric flask. QS to volume with methanol.

17.4.10 Methadone solution 25 µg/mL: Pipet 50 µL methadone standard (1mg/mL) into a 10 mL volumetric flask. QS to volume with methanol.

17.4.11 Calibrators. To 16 x 125 mm screw cap test tubes, add 2 mL blank blood and then add the following amounts of LSD solutions

17.4.11.1 0.100 mg/L = 100 µL of 2 µg/mL LSD stock solution

17.4.11.2 0.050 mg/L = 50 µL of 2 µg/mL LSD stock solution

17.4.11.3 0.010 mg/L = 100 µL of 0.2 µg/mL LSD stock solution

17.4.11.4 0.005 mg/L = 50 µL of 0.2 µg/mL LSD stock solution

17.4.11.5 0.002 mg/L = 20 µL of 0.2 µg/mL LSD stock solution

17.4.11.6 0.001 mg/L = 10 µL of 0.2 µg/mL LSD stock solution

17.4.12 Controls

17.4.12.1 Negative control blood: blood bank blood or equivalent determined not to contain LSD

17.4.12.2 In house LSD control is prepared from a different lot number or different manufacturer of LSD.

17.5 Apparatus

17.5.1 Agilent GC/MSD, Chemstation software, compatable computer and printer

17.5.2 Test tubes, 16 x 125 mm round bottom, screw cap tubes, borosilicate glass with Teflon caps

17.5.3 Test tubes, 16 x 114 mm (10 mL) glass centrifuge, conical bottom

17.5.4 Centrifuge capable of 2,000 – 3,000 rpm

17.5.5 Cleanscreen® Extraction Cartridges (ZSDAU020) from United Chemical Technologies (200 mg columns)

17.5.6 Solid phase extraction manifold

17.5.7 Vortex mixer

17.5.8 Heating block

17.5.9 Evaporator/concentrator

17.5.10 GC autosampler vials and inserts

17.5.11 GC/MSD Parameters. Instrument conditions may be changed or modified to improve performance and sensitivity.

17.5.11.1 Acquisition Mode: SIM (quantitation ions)

17.5.11.1.1 LSD: 221, 323, 181

17.5.11.1.2 LSD d_3: 224

17.5.11.1.3 Mepivicaine: 98

17.5.11.2 Column: HP5 MS 25 m x 0.25 mm x 0.25 µm

17.5.11.3 Detector Temperature: 280° C

17.5.11.4 Oven Program

- Equilibration time: 0.50 minutes
- Initial temp: 130° C
- Initial time: 1 minutes
- Ramp: 17° C/min
- Final Temp: 280° C
- Final Time: 7 minutes
- Run Time: 17 minutes

17.5.11.4.1 Inlet

- Mode: Splitless
- Temperature: 250° C
- Injection volume: 2.0 µL
- Purge Time: ON at 2.0 minute

17.6 Procedure

17.6.1 Label clean 16 x 125 mm screw cap tubes accordingly, blank blood (no IS), negative, calibrators, control(s) and case sample IDs.

17.6.2 Prepare calibrators and controls.

17.6.3 Pipet 2 mL of case samples into appropriately labeled tubes.

17.6.4 Add 4 mL dH_2O to each tube. Vortex briefly.

17.6.5 Add 50 µL LSD-d_3 internal standard to each tube except tube labeled blank (no IS).

17.6.6 Add 20 µL of 25 µg/mL mepivicaine to each tube except tube labeled blank (no IS). Mepivicaine is an alternate internal standard used when LSD-d_3 internal standard contains detectable levels of LSD (assessed by comparing LSD SIM ions in blank blood with and without IS).

17.6.7 Add 20 µL of 25 µg/mL methadone to each vial. Methadone is added as a carrier drug for the SPE extraction.

17.6.8 Add 3.0 mL of pH 6 phosphate buffer to each tube. Vortex for 30 seconds.

17.6.9 Centrifuge at approx 2000 rpm for 10 minutes.

17.6.10 Condition the solid phase extraction columns. Throughout the SPE procedure, it is important not to permit the SPE sorbent bed to dry, unless specified. If necessary, add additional solvent/buffer to re-wet.

17.6.10.1 Add 3 mL hexane to each column and aspirate on vacuum manifold

17.6.10.2 Add 3 mL methanol to each column and aspirate on vacuum manifold.

17.6.10.3 Add 3 mL dH_2O and aspirate.

17.6.10.4 Add 1 mL of 0.1 M pH 6.0 phosphate buffer and aspirate

17.6.11 Without delay, pour specimens into appropriate SPE columns (leaving blood pellet formed during centrifugation in the bottom of each tube). Elute specimens from cartridges with ~ 1-2 mL/ minute flow.

17.6.12 Wash the solid phase extraction columns:

17.6.12.1 Add 3 mL dH_2O and aspirate at ≤ 3inches of mercury.

17.6.12.2 Repeat the dH_2O wash a second time.

17.6.12.3 Wash with 2.0 mL 1.0 M acetic acid and aspirate.

17.6.12.4 Wash with 1 mL methanol. Do not dry.

17.6.12.5 Wipe the SPE column tips with Kimwipes®. Place labeled 10 mL conical test tubes in the manifold test tube rack. Be sure SPE column tips are in the designated conical tube.

17.6.13 Elute drugs by adding 3 mL of freshly prepared dichloromethane/isopropanol/ammonium hydroxide solution to each column. Collect eluate by gravity drain (no vacuum).

17.6.14 Cap conical tubes containing eluate. Refrigerate overnight. If an aqueous layer forms overnight, aspirate aqueous layer.

17.6.15 Evaporate eluates to dryness at approximately 40° C under nitrogen.

17.6.16 Reconstitute samples with 40µL THIA.

17.6.17 Transfer to GC autosampler vials. Inject 2.0 µL on GC/MS in the SIM mode.

17.7 Calculation

17.7.1 Calculate the concentrations by interpolation of a linear plot of the response curve based on ion abundance ratios (using the target ions listed under GCMS conditions) versus calibrator concentration.

17.8 Quality Control and Reporting

17.8.1 See Toxicology Quality Guidelines

17.9 References

17.9.1 United Chemical Technologies, Inc. Clean Screen® solid phase extraction procedure for LSD in serum, plasma or whole blood.